

## Sodium channels as molecular targets in multiple sclerosis

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**Abstract**—Sodium channels are expressed at high density in myelinated axons and play an obligatory role in conducting action potentials along axons within the mammalian brain and spinal cord. It is not surprising, therefore, that they are involved in several aspects of the pathophysiology of multiple sclerosis (MS). First, the deployment of additional sodium channels to demyelinated parts of the axon (which had expressed low densities of sodium channels when covered by the myelin) provides a molecular substrate for the restoration of action potential conduction, a process that contributes to remissions in patients with MS. Second, there is evidence for changes in the expression pattern of sodium channels within Purkinje cells, both in animal models of MS and in human MS. It has been hypothesized that dysregulated sodium channel expression may contribute to symptom production in MS. If this hypothesis is correct, subtype-specific channel blockade may be therapeutically effective as a symptomatic treatment for ataxia and other cerebellar symptoms in MS. Finally, a non-inactivating sodium conductance can trigger calcium-mediated axonal injury via reverse sodium-calcium exchange. Identifying the underlying channel may permit the development of therapeutic strategies that will prevent or retard axonal degeneration in MS.

**Key words:** *action potential, demyelination, molecular plasticity, sodium channels, neuroprotection, nodes of Ranvier, remission, multiple sclerosis.*

### INTRODUCTION

What lessons can we learn from multiple sclerosis (MS) that might help us to design therapies that will preserve or restore function in people with this disorder? Most medications exert their actions on specific target molecules. Clinical observations of spontaneous remissions, in which important functions (e.g., gait, vision, and somatic sensation) are partially or fully restored in the absence of treatment with medications, may point us toward some especially tractable molecular targets in MS. Clinicopathologic observations indicate that, while a small degree of remyelination may occur at the margins of the plaques that are characteristic of MS, remissions can occur in the context of persistent demyelination, i.e., without significant remyelination within the core of the plaques (1–4). In some cases, such as recovery of vision following demyelination of all axons within the optic nerve (5,6), the routing of information along unmyelinated axons or axons that escaped demyelination could not have contributed to functional recovery. While synaptic plasticity at the receiving end may have contributed to functional recovery, useful information must have been conducted as action potentials along some demyelinated axons. Sodium channels are specialized protein molecules that are necessary for

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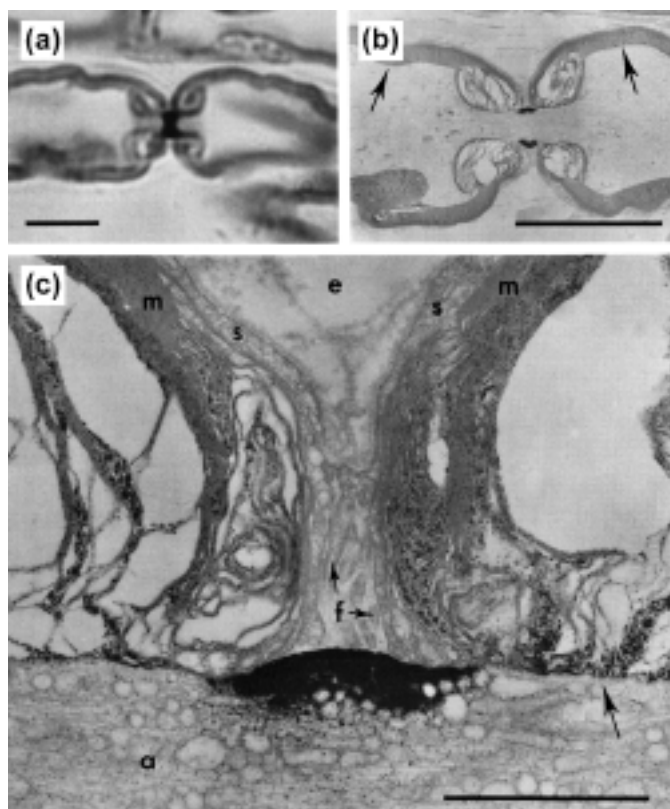
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action potential electrogenesis. Restoration of impulse conduction along demyelinated axons involves a remodeling of the axon membrane, which acquires a higher-than-normal density of sodium channels. Sodium channels thus assume special relevance to restoring function in MS. In this article, we will review what is known about sodium channels in the demyelinating diseases, and we will discuss the implications for the therapeutic manipulation of sodium channels.

### PLASTICITY OF SODIUM CHANNEL EXPRESSION AND RECOVERY OF CONDUCTION IN DEMYELINATED AXONS

Demyelinated axons are elegantly designed. They are much more complex than squid giant axons or nonmyelinated axons, both of which have sodium channels distributed in a homogenous pattern along their length. Within myelinated axons, the sodium channels are clustered in high density (approximately  $1,000/\mu\text{m}^2$ ) within the axon membrane at the node of Ranvier, where they provide the inward current that is required for action potential electrogenesis (**Figure 1**). In contrast, within the internodal axon membrane, i.e., the part of the axon membrane that is covered by the myelin sheath, the density of sodium channels is much lower ( $<25/\mu\text{m}^2$ ) (7–9). The clustered distribution of sodium channels at nodes of Ranvier focuses them where they are needed for saltatory conduction, but at a price: the density of sodium channels in the internodal axon membrane is inadequate for secure conduction of action potentials. Together with the shunting of current that occurs as a result of increased capacitance and conductance of the demyelinated axon membrane and the impedance mismatch that can occur at the boundary between myelinated and demyelinated regions, the low sodium channel density in the demyelinated axon membrane contributes to conduction failure, which produces negative signs and symptoms in the demyelinating diseases (10,11).

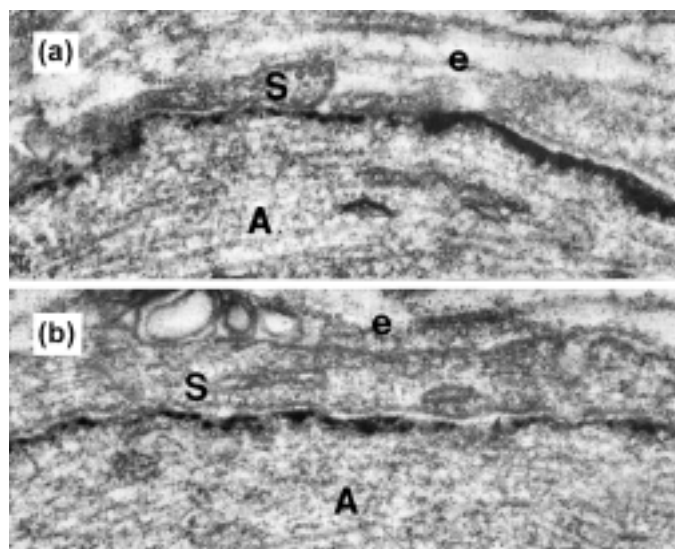
The occurrence of remission following relapses in MS, e.g., demyelination of the optic nerve, implies that action potential conduction resumes in some demyelinated axons. How does this occur? The early electrophysiological observations of Bostock and Sears demonstrated that in some demyelinated axons, continuous conduction of action potentials could be detected as early as 4 to 6 days following demyelination (12,13).



**Figure 1.**

Node of Ranvier stained with ferrocyanide, which provides a marker for regions of high sodium channel density within the axon membrane: (a) shows that light microscopy demonstrates staining of the node of Ranvier; (b) and (c) shows that electron microscopy demonstrates specific staining associated with nodal part of axon membrane. Internodal axon membrane beneath myelin, in contrast, is not stained ( $\rightarrow$ ). a = axon, e = extracellular space, m = myelin, s = Schwann cell cytoplasm, and f = finger-like Schwann cell process. Scale: 10  $\mu\text{m}$ , (a) and (b); 1  $\mu\text{m}$ , (c).

**Figure 2** illustrates the molecular substrate for this restoration of conduction, which depends on the development of diffusely distributed node-like membrane, with high densities of sodium channels, in chronically demyelinated axons (14–16). In addition, “phi nodes,” or hot spots of sodium channels, develop in some chronically demyelinated axons where they can support the development of discontinuous or pseudosaltatory conduction of action potentials through demyelinated regions (17). The involvement of astrocytes, either as sources of sodium channels that are subsequently transferred to axons or as participants in the anchoring of sodium channels in regions of clustering, have both been suggested, but to



**Figure 2.**

Reorganization of demyelinated axon membrane, which acquires a higher-than-normal density of sodium channels. Electron micrograph shows (a) longitudinal section of rat peroneal nerve 16 days after onset of pressure-induced demyelination at a heminode; (b) Dense subaxolemmal staining, similar to that at normal nodes (**Figure 1**), is distributed throughout demyelinated region. A = axoplasm, S = Schwann cell processes, e = extracellular space ( $\times 65,000$ ). Modified from Foster et al. (14).

date, details of the role played by astrocytes in this process remain unknown (18,19).

## MULTIPLE SODIUM CHANNELS

Although traditional neurophysiological doctrine referred to “the” sodium channel as if it were a singular entity, molecular analysis has made it clear that in mammals, there are at least nine different genes, each encoding a distinct voltage-gated sodium channel. Some of these sodium channels are expressed in a highly selective manner, being present in only a small number of types of neurons (20–23). These channels all share a common overall structural motif (24–26). However, the different amino acid sequences of the different channels endow them with different physiological characteristics (e.g., voltage-dependence and kinetic properties) and different pharmacological properties, e.g., sensitivity to tetrodotoxin (TTX). Since sodium channels play an important role in electrogenesis, the selective expression of different ensembles of sodium channels, in different types of

neurons, can endow these cells with different functional properties (27–32). Properties that are critically important for normal neuronal function, such as threshold, refractory period, and action potential patterning, are all affected by the types of sodium channels that are deployed within a given neuron. Thus, the physiological properties of a given neuron depend on the type of channel(s) that are in it, and expression of the wrong types of sodium channels can perturb the functioning of a neuron.

The discovery of this large family of sodium channels raises the questions “Which channels are expressed at the node of Ranvier in normal axons within white matter and which types are expressed in demyelinated axons?” It is now clear that Nav1.6 is the predominant sodium channel at nodes of Ranvier, but the presence of other sodium channels at the node has not been excluded (33,34). Retinal ganglion neurons from the rat, which give rise to axons that are invariably myelinated within the optic nerve, express messenger ribonucleic acid (mRNA) for the Nav1.1, Nav1.2, and Nav1.3 sodium channels, as well as Nav1.6 (35). Consistent with the presence of several types of sodium channels within retinal ganglion neurons, patch clamp studies have demonstrated several sodium currents in these cells (36–38). These observations do not tell us whether other types of sodium channels, in addition to Nav1.6, are deployed at nodes of Ranvier, since it is possible that the additional subtypes are destined for the dendrites or cell bodies of ganglion neurons, or for their central terminals.

A persistent (noninactivating) sodium current, in addition to the rapidly activating, rapidly inactivating sodium current associated with the upstroke of the action potential, can be elicited from myelinated axons within the optic nerve (39). The identity of the channel that produces this persistent sodium current is not yet known. However, a TTX-sensitive, persistent sodium current has been shown to be present within the somata of large dorsal root ganglion (DRG) neurons (40). Large DRG neurons are known to express relatively high levels of the transcripts for the Nav1.6 and Nav1.7 sodium channel (41). Since persistent currents do not appear to be produced by the Nav1.7 sodium channel, Nav1.6 may be responsible for the persistent current. As noted later in this paper, the identification of the channel that produces this persistent current may present a therapeutic opportunity.

The molecular morphogenetic mechanisms responsible for the development and maintenance of sodium

channel deployment in the membrane of myelinated axons are not fully understood at this time. A growing body of evidence suggests that glial-axonal signaling is involved. Early studies suggested that contact with myelin and/or oligodendrocytes suppresses sodium channel expression in the underlying axonal membrane as a result of the presence of specific signaling molecules within the oligodendrocyte and/or myelin membrane (42). Hinson et al. demonstrated that Schwann cells can modulate the transcription of sodium channel genes within DRG neurons in a subtype-specific manner, via both soluble and membrane-associated molecules (43). Boiko et al. have observed that during the development of myelinated axons, Nav1.2 is expressed initially and becomes clustered at immature nodes of Ranvier, but with the advancement of myelination, Nav1.6 replaces Nav1.2 at nodes (44). Kaplan et al. have reported that oligodendrocyte-conditioned medium (presumably because of the presence of soluble oligodendrocyte-derived signaling molecules) can induce clustering of Nav1.2 channels along the central nervous system (CNS) axons (45).

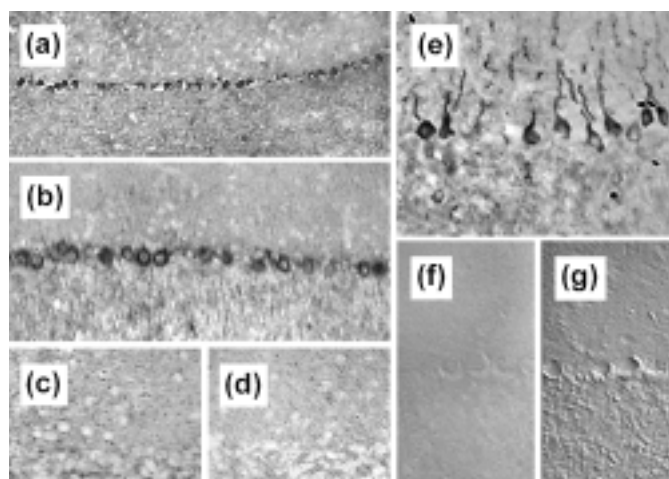
## CHANGES IN DEMYELINATED AXONS

What happens when myelin is lost, as in MS, from a previously myelinated axon? Westenbroek et al. studied the dysmyelinated mutant shiverer in which myelin fails to compact as a result of a defect in myelin basic protein (46); they observed Nav1.2 sodium channels, which are normally not detectable within white matter, along the dysmyelinated white matter axons, a result that has been confirmed by Boiko et al. (44). This result is consistent with the idea that a transition from Nav1.2 to Nav1.6 channels is associated with myelination. However, in the mutant shiverer model, myelin fails to form normally so that demyelination (loss of myelin from previously myelinated axons) cannot be examined.

To address this issue, Black et al. have examined two animal models of myelin loss (one genetic and one inflammatory) and have studied autopsy material from patients with MS (47,48). Black et al. initially studied the mutant taiep rat in which myelin initially develops normally but is subsequently lost because of an abnormality of oligodendrocytes (47,49). They studied expression of the Nav1.8 sodium channel, which is normally detectable only in DRG and trigeminal ganglion neurons, because their earlier studies had demonstrated that

expression of Nav1.8 changes markedly following axonal transection (20,21,50). Using in situ hybridization with Nav1.8-specific probes, Black et al. demonstrated up-regulation of Nav1.8 expression within Purkinje cells after myelin loss within cerebellar white matter in the taiep rat model. Because ion channel synthesis is regulated at both the transcriptional and translational levels, Black et al. also performed immunocytochemical studies using Nav1.8-specific antibodies and observed up-regulated Nav1.8 protein expression in Purkinje cells of taiep rats (47).

More recently, Black et al. examined the expression of Nav1.8 in mice with chronic-relapsing experimental allergic encephalomyelitis (CR-EAE), a widely used inflammatory model of MS, and in humans with MS (48). The CR-EAE model was studied because it provides a model in which consistent demyelinating lesions are within the cerebellum, a feature that is not observed in monophasic EAE (51,52). Using in situ hybridization, Black et al. demonstrated increased expression of Nav1.8 mRNA in Purkinje cells of mice with CR-EAE. Nav1.8 protein was also up-regulated (**Figure 3**) (48). The increased expression of Nav1.8 was not part of a global



**Figure 3.**

The pattern of sodium channel gene expression is altered in EAE, a laboratory model of MS: (a) to (d) show in situ hybridization that demonstrates mRNA expression, and (e) to (g) show immunocytochemistry with subtype-specific antibodies to demonstrate protein expression, for sensory neuron specific (SNS) sodium channel. SNS channels are at much higher levels in Purkinje cells in EAE ((a) and (b), mRNA; and (e), protein) compared to controls ((c), mRNA; (f), protein). (d) = hybridization with sense probes and (g) = Nomarski image of field shown in (f) to demonstrate Purkinje cells ((a),  $\times 100$ ; (b) to (d),  $\times 170$ ; (e) to (g),  $\times 185$ ).

up-regulation of sodium channels within Purkinje cells, because unlike Nav1.8, expression of Nav1.9 (another TTX-resistant sodium channel which, like Nav1.8, is preferentially expressed within DRG and trigeminal ganglion neurons (23)) was not increased.

This up-regulation of Nav1.8 expression is not limited to animal models. Black et al. found that in postmortem brain tissue from patients with disabling secondary progressive MS with demyelination of cerebellar white matter and cerebellar deficits, there was up-regulation of Nav1.8 mRNA and protein expression within cerebellar Purkinje cells (**Figure 4**) (48).

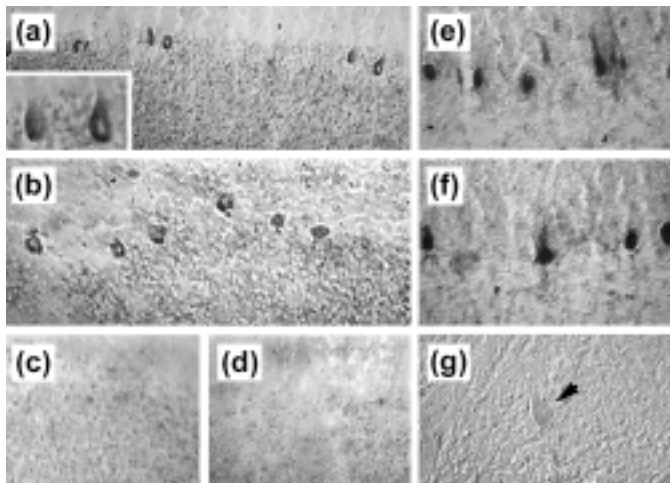
### ADAPTIVE OR MALADAPTIVE?

Is the up-regulation of Nav1.8 in Purkinje cells an adaptive change or a maladaptive one? Consistent with the possibility that Nav1.8 up-regulation may be an adaptive change providing sodium channels that are inserted

into demyelinated Purkinje cell axons where they permit restoration of conduction, Renganathan et al. determined that Nav1.8 channels contribute a substantial fraction of the inward transmembrane current associated with the depolarizing phase of the action potential in the DRG neurons in which these channels are normally expressed (53).

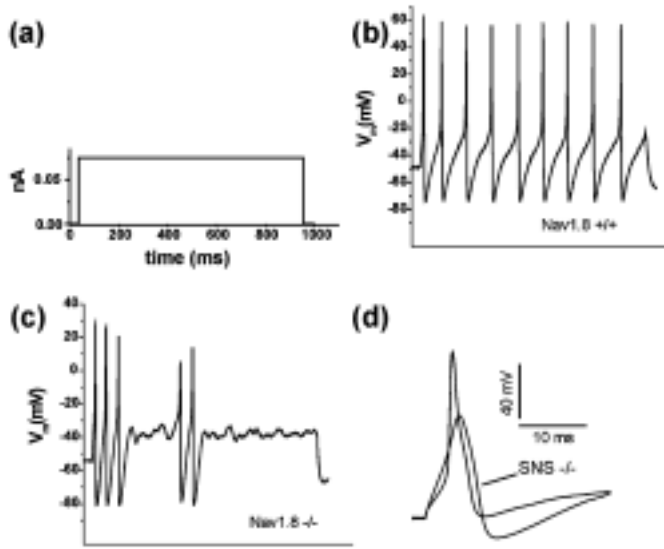
On the other hand, the unique physiological characteristics of Nav1.8, which include slow development of inactivation, a depolarized voltage-dependence of inactivation (20,21), and rapid recovery from inactivation (54,55), suggest that the increased expression of Nav1.8, in Purkinje cells which normally do not express it, may perturb the pattern of electrical activity within the cerebellum. A priori, it might be predicted that the introduction of a different channel, with different voltage dependence and kinetics, might perturb the pattern of Purkinje cell activity. It is well established that the currents produced by sodium channels play a critical role in shaping electrogenesis in these cells (56–58). Mutations of the sodium channels that are present within Purkinje cells have been shown to produce changes in electrogenesis in these cells, which can result in cerebellar ataxia (59,60). In a study on transgenic Nav1.8-null knockout DRG neurons produced by Akopian et al. using transgenic technology, Renganathan et al. used patch-clamp electrophysiology to compare the responses of Nav1.8 (–/–) and Nav1.8 (+/+) DRG neurons with identical electrical stimuli, and observed differences in the pattern of action potential firing and in the shape of action potentials (this latter change might, in turn, affect activation of N-type calcium channels, thereby altering the transmitter release if Nav1.8 is present at axon terminals) (**Figure 5**) (28,53,61).

Black et al. have proposed that altered patterns of electrical activity within cerebellar circuits, caused by abnormal Nav1.8 expression within Purkinje cells, may contribute to ataxia and other cerebellar symptoms in MS (48). This hypothesis, which implicates Nav1.8 as a potential molecular target in MS, is currently being tested in laboratory models. A number of research groups are attempting to develop Nav1.8-specific sodium channel-blocking drugs. When these are available, the next step will be to study the effects of Nav1.8 blockade on cerebellar deficits in animal models of MS and ultimately in humans with MS.



**Figure 4.**

Up-regulated expression of SNS sodium channels within cerebellar Purkinje cells from brains obtained at postmortem from MS patients: (a) to (d) on left show in situ hybridization with SNS-specific antisense riboprobes and demonstrate up-regulated expression of SNS mRNA within Purkinje cells in postmortem tissue from two patients with MS (a) and (b) compared to controls (c). In (d), no SNS mRNA signal is detectable following hybridization with sense riboprobe. (e) to (g) on right show immunostaining with an antibody directed against SNS and illustrate up-regulated SNS protein in Purkinje cells from patients with MS (e) and (f) compared to control cerebellum (g), ← indicates Purkinje cell ((a),  $\times 75$ ; inset,  $\times 185$ ; (b) to (d),  $\times 100$ ; (e) to (g),  $\times 150$ .) Modified from Black et al. (48).



**Figure 5.**

Altered expression of SNS sodium channel gene can influence pattern of action potentials and action potential configuration: Firing pattern in response to (a) identical depolarizing stimulus is different in (b) SNS +/+ DRG neuron compared with (c) SNS -/- DRG neuron from a knockout mouse. (d) shows action potential configuration is also different in Nav1.8 +/+ and Nav1.8 -/- neurons. Modified from Renganathan et al. (53).

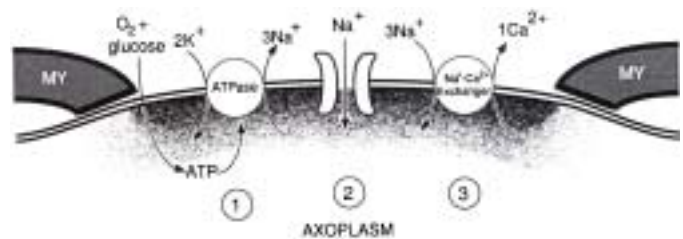
## AXONAL DEGENERATION IN MS: ROLE OF SODIUM CHANNELS

What are the molecular mechanisms that produce axonal degeneration in MS? In addition, can it be prevented? Since axonal degeneration has been associated with developing permanent nonremitting deficits in MS (62–66), this is an important goal. “Neuroprotection” has received considerable attention from neuroscientists, but the great majority of studies on it deal with gray matter, such as the cerebral cortex and basal ganglia. Within gray matter, excitotoxicity appears to play a significant role in neuronal injury. However, it would be expected a priori that excitotoxicity should not injure axons, since they do not express the receptors for excitatory neurotransmitters. Ransom et al., in fact, demonstrated that high levels of glutamate do not injure axons within CNS white matter (in contrast to oligodendrocytes, which express glutamate receptors and can be injured by glutamate) (67).

If CNS axons within CNS white matter are not injured by excitotoxic mechanisms, how do they die? Does calcium-mediated injury occur within white matter, and if so, what mechanisms trigger it? Stys et al.

addressed these questions using the anoxic optic nerve as a model (68). They showed that calcium-mediated axonal injury within CNS white matter is mediated by reverse operation of the sodium-calcium exchanger which, in turn, is driven by a reduction in the transmembrane sodium gradient and depolarization, both caused by sodium influx via a TTX-sensitive sodium conductance. As shown in **Figure 6**, the results of Stys et al. indicate that anoxia triggers ATPase failure within the optic nerve and a depolarization of axon results. This activates sodium channels that act as a route for a persistent sodium influx that collapses the transmembrane gradient for sodium. These events, in turn, trigger reverse operation of the sodium-calcium exchanger, resulting in an increase in intracellular calcium, which injures the axon (68).

The schema presented in **Figure 6** suggests the presence, in myelinated axons of the optic nerve, of a persistent sodium conductance. Using grease-gap-recording methods, Stys et al. demonstrated that, in fact, a persistent sodium conductance is present within optic nerve axons (39). The molecular identity of the sodium channel responsible for this conductance is not yet known. A clue may lie in the presence of a low-threshold persistent sodium current in large DRG neurons; although the identity of the underlying channel has not yet been delineated, Baker and Bostock demonstrated that this current is TTX sensitive (40). This indicates that the TTX-resistant sodium channels that have been cloned from DRG



**Figure 6.**

Molecular cascade leading to calcium-mediated injury of axons within CNS white matter. ① ATP depletion can lead to failure of Na<sup>+</sup>/K<sup>+</sup> ATPase, which results in depolarization and collapse of transmembrane ionic gradients. ② Na<sup>+</sup> ions enter axon via persistent sodium conductance, further contributing to loss of the transmembrane Na<sup>+</sup> gradient. ③ Resultant increase in intracellular sodium, together with depolarization, triggers reverse sodium-calcium exchange. Increased intracellular calcium levels activate deleterious events, which contribute to axonal degeneration (Stys et al. (68)).

neurons (including Nav1.9/NaN, which is known to produce a persistent sodium current (29)) are not candidates. Of the TTX-sensitive sodium channels, Nav1.6 and Nav1.7 are known to be expressed at relatively high levels within large DRG neurons (41). Patch clamp studies on Nav1.7 indicate that it does not produce a persistent current (27). Thus, Nav1.6 must be considered as a potential source of this current.

Definitive identification of the channel underlying the persistent sodium current in optic nerve axons may be therapeutically important, since subtype-specific sodium-channel-blocking drugs may soon become available. The relevance of the cascade shown in **Figure 6** to MS is underscored by the suggestion that ischemic injury may occur in MS (69). In addition, even in the absence of ischemic injury, nitric oxide (NO), which is present at injurious levels within MS lesions, may produce mitochondrial injury, thereby leading to a reduction in ATP levels (70–73). NO-mediated blockade of conduction in demyelinated axons (74,75), possibly because of the blockade of sodium channels by NO (76,77), might indicate that NO's actions include a protective effect. This would appear, however, to be eclipsed by an opposing deleterious effect of NO on axonal energy metabolism, which can result in axonal degeneration. Consistent with this suggestion, several studies have reported that NO, when applied to a white matter tract, can trigger axonal degeneration (78,79). Irrespective of whether the triggering event involves ischemia, NO, or other factors, if a sodium channel permits a persistent sodium influx that drives a reverse sodium-calcium exchange in CNS axons in MS, pharmacological blockade of this channel might retard, or prevent, axonal degeneration, thus preserving function.

## CONCLUSIONS

As outlined in the previous paragraphs, sodium channels most likely play important roles in several aspects of the pathophysiology of MS. Details of their roles are not yet fully understood, but importantly, the molecular structures of nine different sodium channels are now known and an increasing body of evidence implicates specific subtypes of sodium channels in the pathophysiology of MS. In view of their distinct amino acid sequences, each subtype may be amenable to a specific blockade that leaves other sodium channel subtypes

unaffected, thereby limiting side effects. Although much work remains to be done, the results obtained thus far suggest that pharmacologic manipulation of sodium channels may provide some new therapeutic strategies for MS.

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